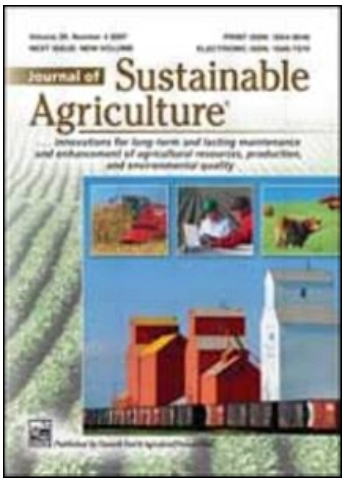

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Effect of Wild Radish on Preimaginal Development of *Diabrotica balteata* and *Agrotis ipsilon*

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Aqueous extracts of wild radish (Rhaphanus raphanistrum L.) roots, stems, and leaves combined were examined for antibiotic effects on hatching and larval development of the banded cucumber beetle, (Diabrotica balteata Le Conte [Coleoptera: Chrysomelidae]) and black cutworm (Agrotis ipsilon [Hufnagel] [Lepidoptera: Noctuidae]). Hatching was delayed significantly in eggs of D. balteata exposed to the wild radish extract for 18 h. In some trials, the hatching percentage increased to levels similar to other treatments by the third and final day of hatching; in others, the percentage hatching remained significantly less among eggs exposed to wild radish extract for the duration of the study. The mortality rate of D. balteata larvae was increased significantly by exposure to the wild radish extract. Hatching of A. ipsilon eggs was delayed significantly after an 18 h exposure to the aqueous wild radish extract 4 d after treatment in only one of the six trials. In that trial, all treatment results were similar by day 5. In other trials, hatching of A. ipsilon was decreased similarly in eggs exposed for 18 h in an aqueous wild radish treatment as well as the water control. Although this study shows an effect of aqueous wild radish extract on two insect

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species, supplemental studies which utilize methods that do not rely upon an aqueous solution may better define the insect suppression activity of wild radish.

KEYWORDS black cutworm, corn, corn rootworm, pest suppression, preimaginal development, vegetables, wild radish

INTRODUCTION

Wild radish (*Rhaphanus raphanistrum* L.[Brassicaceae]) is prevalent throughout the Southeastern United States. It produces weed suppressant chemicals which reduce sicklepod, pitted morningglory, and prickly sida emergence and shoot fresh weight (Norsworthy, 2003). In recent research, desiccated wild radish was found to have a negative impact on strip-tilled cotton stands and resulted in over 90% stand reduction (Norsworthy, unpublished data). However, in that research, wild radish had no effect on strip-tilled field corn or no-till, drill-seeded soybean stands 4 weeks after emergence.

Pest insect feeding of *Microtheca punctigera* (Achard) (Coleoptera: Chrysomelidae) on wild radish was 25% and 28% of that found on mustard (*Brassica juncea* [L.] Czern. & Cosson) and Chinese cabbage (*B. pekinensis* [Lour.] Rupr.), respectively, although significant differences were not detected. It is probable that the insect was attracted to the plant, but the presence of arrestants or lack of feeding stimulants caused relatively weak feeding (Menezes et al., 2005). In other research, Williams et al. (1995) and Borek et al. (1995) detected isothiocyanates in most brassicaceous plants that were toxic to the black vine weevil (*Otiorhynchus sulcatus* [F.]). The relative growth rates of two lepidopterous pests, the diamondback moth (*Plutella xylostella* [L.]) and southern armyworm (*Spodoptera eridania* [Cramer]), were lower on lines of *B. juncea* with high glucosinolate concentrations [6.8 – 21.3 μg/g (FW)] (Li et al., 2000). In toxicity experiments with artificial diets, isothiocyanate and glucosinolates were lethally toxic to neonate *S. eridania*. The plant chemicals also play a role in the development of insect defense mechanisms (Aliabadi et al., 2002). Some species of crucifers can be toxic to some organisms. The harlequin bug, *Murgantia histrionica* (Hahn), is a pentatomid that feeds on crucifer plants that are toxic to some organisms, and it can sequester and retain glucosinolates from its host plant as a defense mechanism against predaceous birds (Aliabadi et al., 2002). Larvae of the large white butterfly, *Pieris brassicae* (L.), feed on crucifers, resulting in the development of toxic adults (Aplin et al., 1975). Moreover, chemicals in some *Brassica* species can have an impact on the biology of certain arthropods (McCloskey and Isman, 1993; Huang and Renwick, 1994;

Traynder and Truscott, 1991). Francis et al. (2001) linked biological parameters of aphid predators with the chemical composition of *Brassicaceae*. Blau et al. (1978) reported that *Pieris rapae* (L.), a crucifer specialist, is not affected by artificially high concentrations of allylglucosinolates. However, larval growth of *Spodoptera eridania*, a generalist feeder, is inhibited by high, but not by low concentrations of allylglucosinolates. Our previous research indicates that exposure to flowers of wild radish decreases survival and longevity of a parasitic wasp, *Diadegma insulare* (Cresson), which attacks and kills the diamondback moth (*P. xylostella*), a major pest of collard and cabbage (Gourdine et al., 2005). We report the results of laboratory bioassays which were conducted to determine the effect of an aqueous wild radish extract on egg hatching and larval development on two economically important corn pests, *Diabrotica balteata* LeConte (banded cucumber beetle, BCB) and *Agrotis ipsilon* (Hufnagel) (black cutworm, BCW).

MATERIALS AND METHODS

Whole plants (roots, leaves, and stems) of wild radish were harvested, oven-dried, ground and passed through a 1 mm sieve. Cold distilled water and oven-dried ground wild radish were mixed in a ratio of 10 ml to 1 g (v/w), using 30 mL water and 3 g wild radish. The mixture was left to stand for 0.5 h. Coarse particles were strained with organdy over a 50 mL flask. Finer particles were removed by straining again with filter paper (grade 36).

Diabrotica balteata eggs that were deposited by moths on paper towels and 6-day-old *D. balteata* larvae were obtained from a colony maintained at the USDA, ARS, U.S. Vegetable Laboratory in Charleston, SC. Larvae were reared at room temperature on sprouted wheat soaked in Captan® to inhibit fungi. Five *D. balteata* eggs were transferred to each 30 ml cup onto strips of filter paper (1.0 cm × 2.0 cm) soaked with the designated treatment. Five treatments were tested: 1) eggs soaked in wild radish extract for 18 h, 2) eggs soaked in distilled water for 18 h, 3) control in which eggs were transferred directly from the paper towel substrate, 4) eggs soaked in distilled water for 0.5 h, and 5) eggs soaked in wild radish extract for 0.5 h. The treatments were replicated 24 times with 120 experimental units consisting of 600 eggs in each of 4 trials. Percentage hatch was recorded from the first day of hatching and for each of the next 3 to 4 days.

Eggs of *A. ipsilon* were purchased from Benzon Research (Carlisle, PA). A laboratory colony was subsequently maintained by placing neonate larvae on artificial diet (multi-species insect diet). Upon pupation, they were placed in 3.8 L wide-mouth glass jars with cheese cloth (20 cm × 8 cm) which was hung from the rim for an oviposition substrate. The eggs were

tested in the five treatments as described above. Treatments were replicated 24 times in each of 7 trials.

To conduct larval bioassays, the wild radish filtrate (1.5 mL) was used to saturate a filter paper (grade 36, 9 cm diameter, folded in half twice) in each 30 mL cup. The filtrate was made by mixing 100 g wild radish with 450 mL water (100% extract) and 100 g wild radish with 900 mL water (50% extract). A single 6-day-old larva of *D. balteata* and two sprouted wheat seeds or a second instar *A. ipsilon* and one gram multi-species artificial insect diet were placed in 30 mL plastic cups that had been treated with either 100% extract, 50% extract, or water for the control. The plastic cups were covered with a plastic lid. The three treatments were replicated 50 times with 150 experimental units testing 150 BCB and BCW larvae in each of four trials. The test insects were placed in an environmental chamber at 25 ± 2° C, 80 ± 2% RH, and 14:10 L:D regimen. Larvae were checked daily, and mortality data were recorded. The data were subjected to a randomized block design and then to analysis of variance (ANOVA) for separation of means with least significant difference (LSD) (Analytical Software 2000).

RESULTS AND DISCUSSION

Hatching of *D. balteata* eggs was first observed 7 days after treatment (DAT) in the control and 18 h water treatments. Hatching was delayed by the wild radish extract treatment in which eggs were exposed for 18 h in each of the four laboratory trials (Figure 1a-d). Statistical results of the affect of wild radish at the onset of hatching of *D. balteata* are depicted in Table 1. Percent hatch of eggs in the 18 h water treatment was similar to the untreated control throughout the study. Percentage hatch of eggs in both the 0.5 h water and 0.5 h wild radish treatments were also similar. Mean percentage hatch of *D. balteata* eggs exposed to various treatments across all trials ranged from 0.0 to 62.5 for 7 DAT, 0.0 to 80.8 for 8 DAT, 5.0 to 83.3 for 9 DAT, and 61.7 to 78.3 for 10 DAT. Percentage hatch was significantly less in the 18 h wild radish treatment than in all other treatments for the duration of the experiment in two of the four trials (t = 12.32 and 12.67, Pr > t = 0.050). When *D. balteata* eggs are deposited in the field in cracks in the soil, they require 5 to 9 days to hatch (Marsh, 1912). This range is affected by environmental factors. There may be opportunities to adjust these factors with the use of cover crops. The effect of wild radish on the hatching of eggs of *D. balteata* may be important in the pest management decision-making process. To better define the importance of the wild radish factor, a field test should be designed to provide exposure of *D. balteata* to wild radish extract for 18 h.

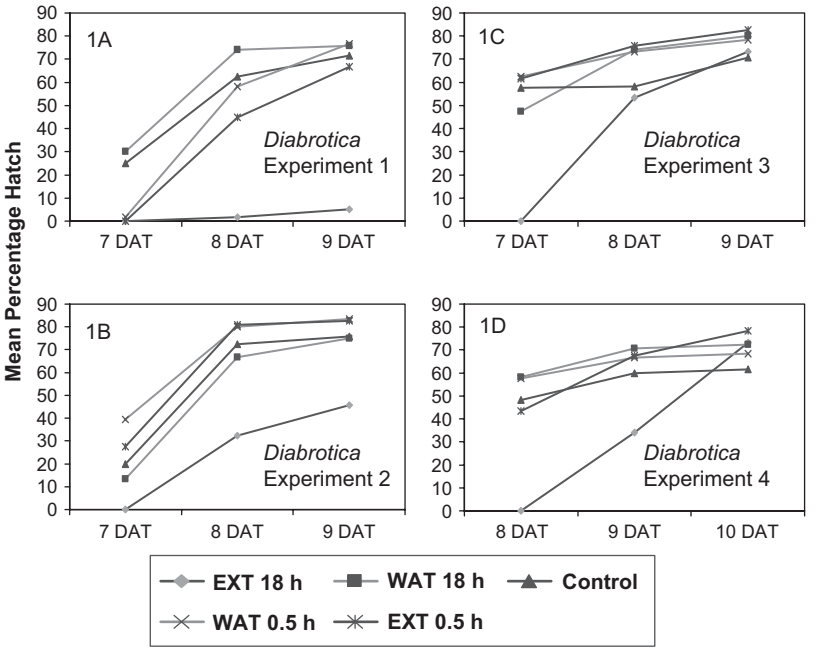

FIGURE 1 Mean percentages of egg hatching by *D. balteata* over selected days when exposed to different treatments of wild radish extracts in the laboratory. EXT 18 h = 18 h exposure to the extract; EXT 0.5h = 0.5 h of exposure to the extract; WAT 18 h = 18 h of exposure to water; WAT 0.5 h = 0.5 h of exposure to water; Control = untreated.

TABLE 1 Mean percentage hatch by *D. balteata* exposed to extracts of wild radish (WRAD) in laboratory bioassays after 10 days

Treatment	Trial 1	Trial 2	Trial 3	Trial 4
18 h WRAD	0.0 c	0.0 c	0.0 c	0.0 c
18 h Water	30.0 a	13.3 b	47.5 b	58.3 a
Control	25.0 a	20.0 ab	57.5 ab	48.3 b
0.5 h Water	1.7 b	27.5 a	61.7 a	57.5 ab
0.5 h WRAD	0.0 c	29.6 a	62.5 a	48.3 b
SE =	4.88	5.03	6.25	7.57

Means in the same column followed by different letters are significantly different ($p < 0.05$).

There was an effect on *D. balteata*, but there was no mortality by *A. ipsilon* larvae after they were exposed to wild radish extracts. The mortality rate of *D. balteata* larvae was highest (about threefold) in 100% extract, as compared with the 50% extract and the control samples (Figure 2). However, these data were not consistent from trial to trial. In the other three trials, there were no differences in mortality rate with the various concentrations of aqueous extract.

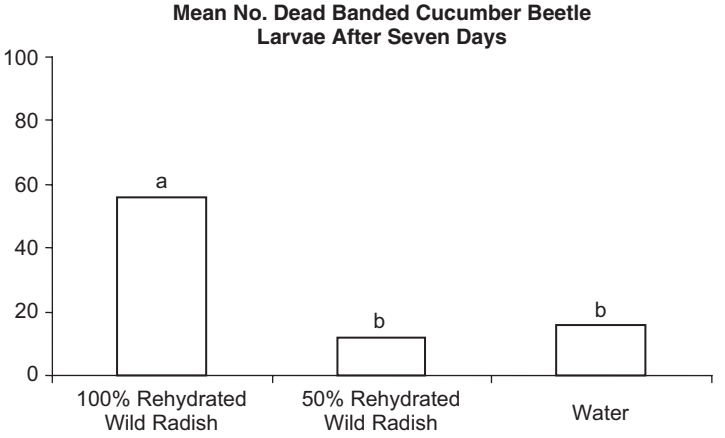

FIGURE 2 Mean number of dead banded cucumber beetle larvae after 7 days of exposure to selected percentages of rehydrated wild radish extract in the laboratory.

Response of black cutworm eggs to exposure to wild radish extract was different from that of the banded cucumber beetle. Hatching generally occurred 4 DAT (Figure 3a-g). Hatching of the black cutworm was significantly delayed in only one of the six trials 4 DAT (Figure 3-f). However, by the next day (5 DAT) in that trial, percent hatching for all treatments was similar (t = 10.07, Pr > t = 0.050). Larvae of the black cutworm apparently exhibited tolerance to the wild radish and even appeared to flourish. All larvae that hatched from eggs exposed to wild radish were alive after 4, 6, and 10 d in the environmental chamber. They pupated in another 3 d, and adult moths emerged 12 d after pupation. Busching and Turpin (1977) reported that the larval stage of *A. ipsilon* ranged from 24.6 d on wheat to 47 d on morningglory (*Ipomoea* spp.) at 26.7 °C (day):15.6 °C (night) in a 15:9 L:D regimen. Hence, the wild radish had no apparent negative effect on development of the black cutworm under the described laboratory conditions. Even though hatching in the wild radish 18 h treatment was significantly less than the control, it was similar to the 18 h water treatment in the first four trials (Table 2). Therefore, there is little evidence that glucosinolate products from wild radish can disrupt the development of black cutworm. Hillyer and Thorsteinson (1969) reported that glucosinolates may stimulate oviposition in the diamondback moth [*Plutella xylostella* (L.)], a specialist on brassicas. We report a negative effect on hatching of the black cutworm by the extract and by the water treatments when eggs were exposed for 18 h. It is important to recognize that extended exposure to moisture is not a part of the normal rearing process of *A. ipsilon*. The extended exposure to aqueous treatments was detrimental to the hatching of *A. ipsilon* eggs. The 18 h water treatment was less than all other treatments in only one trial.

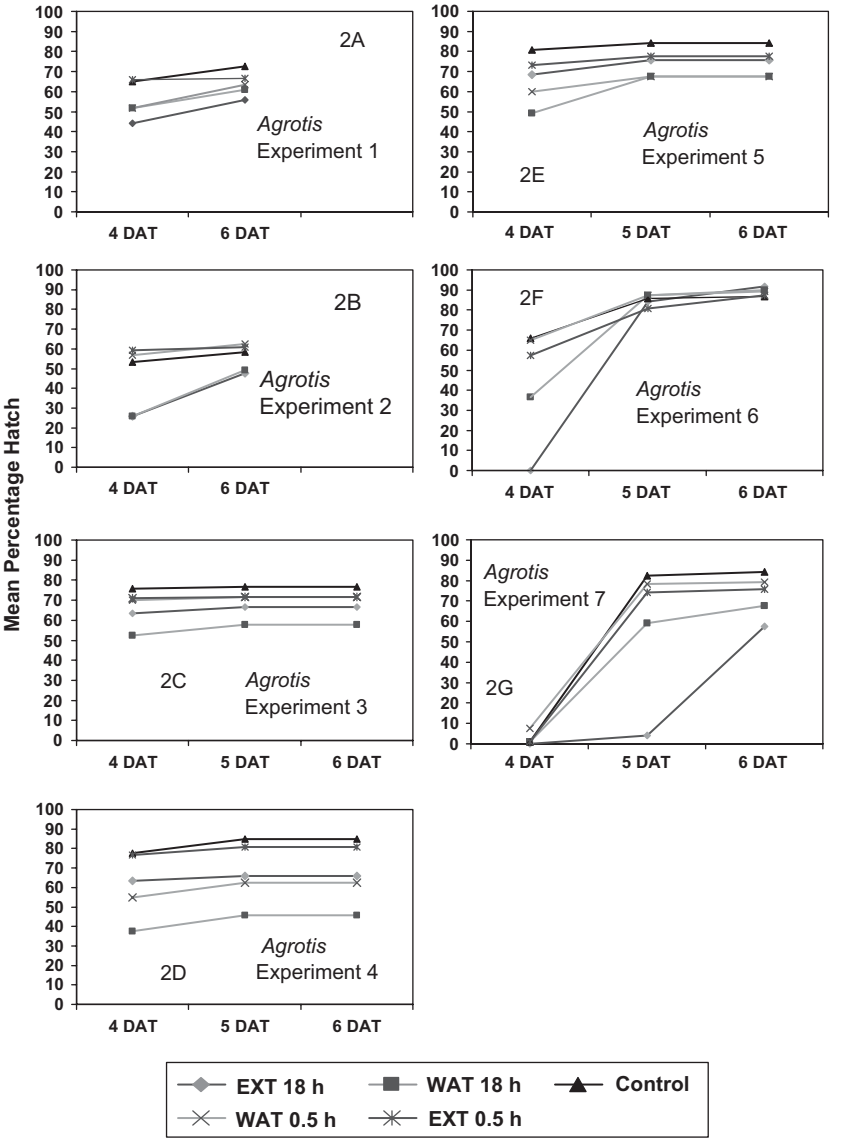

FIGURE 3 Mean percentages of egg hatching by *A. ipsilon* over selected days when exposed to different treatments of wild radish extracts in the laboratory. EXT 18 h = 18 h exposure to the extract; EXT 0.5 h = 0.5 h of exposure to the extract; WAT 18 h = 18 h of exposure to water; WAT 0.5 h = 0.5 h of exposure to water; Control = untreated.

We collected additional data from sweet corn planted in four-row field plots after wild radish and rye cover crops (unpublished data). In 2004 and 2005, the plots were planted in South Carolina using a split-plot design with main plots of cover crops and weed management programs, including weedy check, hand weeded check, and 0.5X and 1X rates of *S*-metolachlor

TABLE 2 Mean percentage hatch by *A. ipsilon* exposed to extracts of wild radish (WRAD) in laboratory bioassays after 10 days

Treatment	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
18 h WRAD	44.2 b	25.8 b	63.3 bc	63.3 bc	68.3 bc	0.0 c
18 h Water	51.7 b	25.8 b	52.5 c	37.5 c	49.2 d	36.7 b
Control	65.0 a	53.3 a	75.8 a	77.5 a	80.8 a	65.8 a
0.5 h Water	51.7 b	56.7 a	70.0 ab	55.0 b	60.0 cd	65.0 a
0.5 h WRAD	65.8 a	59.2 a	70.8 ab	76.7 a	73.3 ab	57.5 a
SE =	6.39	6.54	5.87	6.60	5.80	5.58

Means in the same column followed by different letters are significantly different ($p < 0.05$).

plus atrazine (Bicep II Magnum®), as subplots. When population densities ranged from 0.3 to 2.2 corn earworms (*Helicoverpa zea* Boddie) per corn ear in the plots in 2004, there were more earworms per corn ear in the wild radish cover treated with herbicides than in the weedy checks in rye and no cover crop as well as in no cover crop treated with 0.5X Bicep II Magnum®. However, in 2005, when population density was much lower (<0.5 corn earworm per corn ear in all treatments), there were no differences among treatments. Data were inconsistent with corn earworm populations from year to year in the various cover crops. Yield was higher in wild radish treatments in 2004, and there were no consistencies in determining differences among cover crop treatments in 2005 when marketable ears were examined. The history of crops and weeds may be important over a long period and in large test plots to determine effect of wild radish on populations of soil borne insects. Although the laboratory data demonstrated an impact, additional field studies are needed to ascertain the magnitude of the effect of wild radish on insect pest populations.

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